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Congruent Evolution Between Whiteflies (Homoptera: Aleyrodidae) and Their Bacterial Endosymbionts Based on Respective 18S and 16S rDNAs

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Abstract. Whiteflies (family Aleyrodidae) possess heritable eubacterial endosymbionts sustained in specialized organ-like structures called mycetomes. Comparisons of distances between the ash whitefly, *Siphoninus phillyreae,* and two biotypes ("A" and "B") of the sweetpotato whitefly, *Bemisia tabaci,* based on sequence analysis of genes for 18S rRNAs (rDNAs), were equivalent to the distances represented by the 16S rDNAs of their respective endosymbionts. This finding indicates that evolutionary divergence in whitefly hosts and their endosymbionts is congruent. The nucleotide sequences of the 18S rDNAs and endosymbiont 16S rDNAs indicate the two biotypes of *B. tabaci* are the same species.

The whiteflies (family Aleyrodidae) are a taxonomically distinct group of plant sap-feeding insects placed in the homopteran suborder Sternorryncha, which includes the aphids, mealybugs, and scales [12]. Whitefly biology is comparable to that of aphids and mealybugs. However, whiteflies are considered to be essentially tropical, whereas aphids and mealybugs are common to temperate climates [5, 10].

Like aphids and mealybugs, whiteflies maintain an obligate relationship with symbiotic eubacteria (endosymbionts) that are harbored in specially adapted polyploid cells termed mycetocytes [4, 19]. Recently, molecular phylogenetic analysis of eubacterial 16S rRNA genes (16S rDNA) revealed that the primary endosymbionts of whiteflies evolved from a lineage distinct from those of the endosymbionts of aphids and mealybugs [7]. The primary endosymbionts of whiteflies form a distinct clade outside the γ-3 subdivision of the *Proteobacteria*. In contrast, the endosymbionts of aphids and mealybugs form monophyletic clades within the γ-3 subdivision [14] and β subdivision [15], respectively.

A few species of whiteflies have become significant agricultural pests in the United States. Record crop losses in 1981 in California and Arizona were caused by a biotype (designated "A" or "cotton") of the sweetpotato whitefly, *Bemisia tabaci,* which transmitted a number of phytopathogenic viruses [9]. Recently, a more polyphagous and phytotoxic biotype (designated "B" or "poinsettia") of *B. tabaci* has spread from Florida across the Southern United States to California, resulting in severe losses of both agricultural and ornamental crops [3, 8, 16]. The ash whitefly, *Siphoninus phillyreae,* was a major pest of both horticultural and agricultural crops in the late 1980s and early 1990s throughout California. It has recently been brought under control by a commendable pest management program that relied mostly on parasitic wasps and predaceous beetles [2].

In a previous study, distinct differences were found between the nucleotide sequences of 16S rDNAs of the endosymbiont of *B. tabaci* and *S. phillyreae,* whereas the 16S rDNAs of the A and B biotypes of *B. tabaci* were indistinguishable [7]. In the present study, a fragment of approximately 1050 base pairs (bp) of the back portion of the 18S rRNA gene of each of these whiteflies was cloned and sequenced. The results show that the distances between the 18S rDNAs of the whitefly hosts are equivalent to previously published [7] distances between the 16S rDNAs of their respective endosymbionts.

Materials and Methods

The A and B biotypes of *B. tabaci* were provided by J.E. Duffus (USDA-ARS, Salinas, California) and L.S. Osborne (University of Florida, Apopka, Florida). Ash whiteflies were collected from

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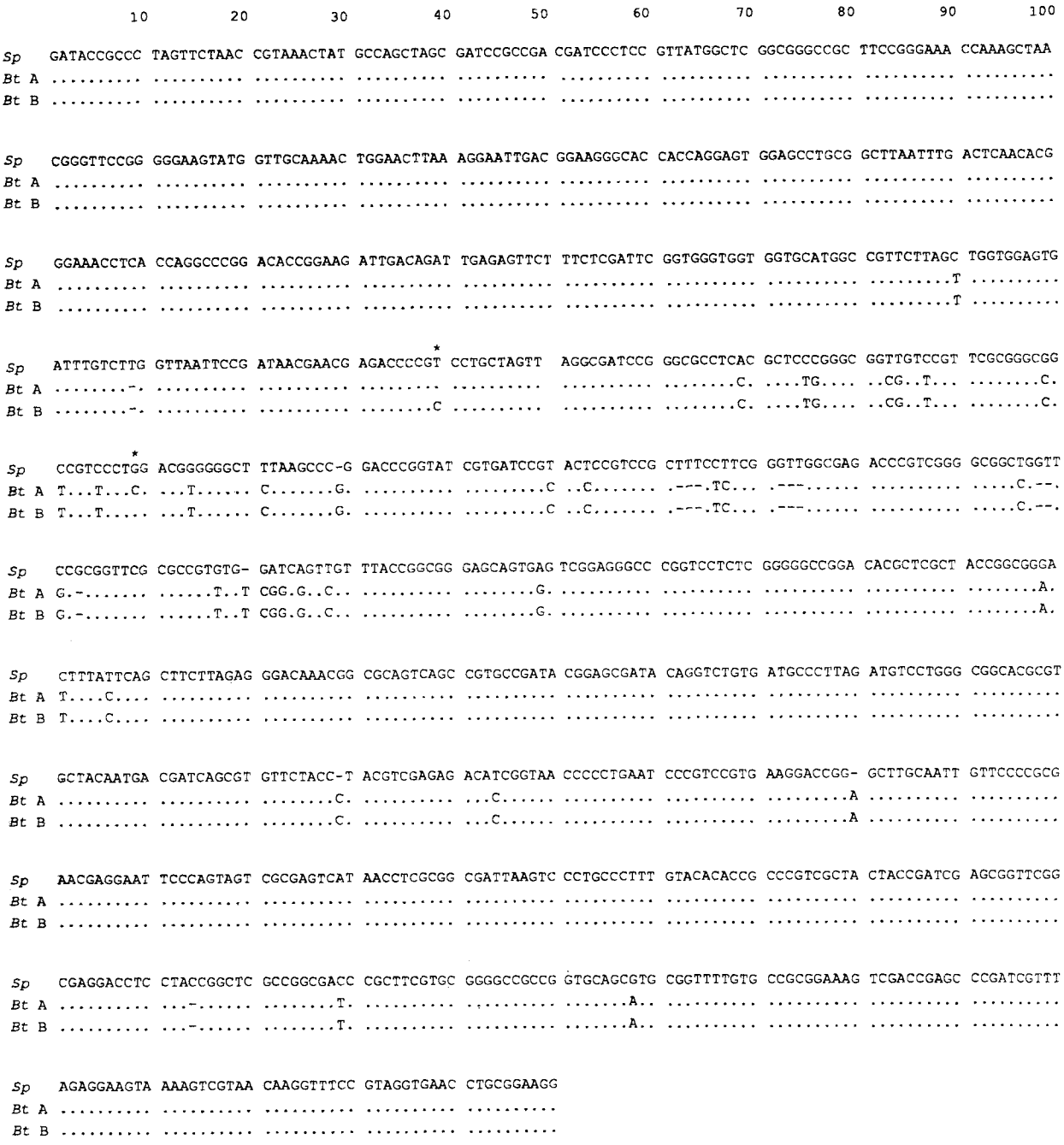

Fig. 1. Aligned nucleotide sequences of the back fragment (approx. 1050 bp) of the 18S rRNA genes of *Siphoninus phillyreae* (*Sp*) and the "A" (*Bt* A) and "B" (*Bt* B) biotypes of *Bemisia tabaci*. Only mismatches between *Sp* and *Bt* A and B are shown for *Bt* A and B (* indicates mismatch between *Bt* A and *Bt* B).

citrus and apple trees in Concord, California. DNA was purified from the whiteflies according to methods outlined previously [7].

A back portion of the 18S rDNA of each whitefly was amplified by the polymerase chain reaction (PCR) [17] according to methods previously described [6]. The following PCR primers were used: forward 5′-GAT ACC GCC CTA GTT CTA ACC-3′, and reverse 5′-TCC TTC CGC AGG TTC ACC-3′ [equivalent to positions 1067–1087 and 1927–1944, respectively, of the 18S rRNA gene of the mosquito *Aedes albopictus* (1)]. Cloning and sequencing (both strands) of the amplified 18S rDNA were performed as previously described [6]. Nucleotide sequences of the 18S rDNA fragments of these whitefly taxa were aligned by use of the GeneWorks® ver 2.1 (Intelligenetics, Mountainview, California) computer program for the Macintosh.

Results and Discussion

PCR amplification, cloning, and sequencing. PCR amplification with the above primers of 18S rDNAs yielded a singular band of 1039 bp for each biotype of *B. tabaci* and 1047 bp for *S. phillyreae*. These back fragments of the whitefly 18S rDNA were approximately 30 bp smaller than the predicted corresponding fragment of the pea aphid, *Acyrthosiphon pisum*, the only other published sequence of a homopteran 18S rDNA [13]. The whitefly 18S rDNA fragments were, however, significantly larger than the corresponding fragments predicted from the 18S rDNAs of the following nonhomopteran insects: *Drosophila melanogaster* [903 bp (18)], *Aedes albopictus* [877 bp (1)] and *Tenebrio molitor* [851 bp (11)]. The aligned sequences of the back fragments of the whitefly 18S rDNAs are presented in Fig. 1 and are deposited with EMBL, accession numbers Z15051–Z15053.

Comparisons between host 18S and endosymbiont 16S rDNAs. Only two base differences were found in a comparison of the nucleotide sequences of the 1039 bp fragments of the 18S rDNAs of the A and B biotypes of *B. tabaci*. An identical number of bases differed between the 16S rDNAs (1551 bp) of the primary endosymbionts of these two strains [7]. The two base differences in both cases are probably PCR anomalies. The 18S rDNA fragments of both biotypes of *B. tabaci* differed from that of *S. phillyreae* by 46 bases for a calculated percentage distance of 4.43. The 16S rDNAs of the primary endosymbionts of *B. tabaci* and *S. phillyreae* differed by 70 bases for a calculated percentage distance of 4.51 [7]. Thus, the distances between each of the taxa revealed by examination of the back fragment of their 18S rDNAs were essentially identical with those demonstrated by the 16S rDNAs of their primary endosymbionts.

Conclusions

(1) Host and endosymbiont rDNAs reveal similar characteristics with regard to the evolutionary relationship between the whitefly taxa examined in this study. This finding suggests that the molecular evolution of both genes is synchronous.

(2) This synchronization implies that endosymbiont 16S rDNAs can be reliable indicators of phylogenetic relationships between their host insects within the Homoptera. This conclusion is in agreement with earlier findings which showed that the phylogenetic relationship of aphid endosymbionts was congruent with the classical systematic placement of their hosts [14].

(3) The identical sequences of host 18S rDNAs and their respective endosymbiont 16S rDNAs is consistent with the biotypes of *B. tabaci* being the same species.

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Literature Cited

1. Baldridge GD, Fallon AM (1991) Nucleotide sequence of a mosquito 18S ribosomal RNA gene. Biochim Biophys Acta 1089:396–400
2. Bellows TS, Paine TD, Gould JR, Bezark LG, Ball JC (1992) Biological control of ash whitefly: a success in progress. Calif Agric 46:24–27
3. Bethke JA, Paine TD, Nuessly GS (1991) Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsetta. Ann Entomol Soc Am 84:407–411
4. Buchner P (1965) Endosymbiosis of animals with plant microorganisms. New York: Interscience Publishers, Inc., pp 210–338
5. Byrne DN, Bellows TS (1991) Whitefly biology. Annu Rev Entomol 36:431–457
6. Campbell BC, Bragg TS, Turner CE (1992) Phylogeny of symbiotic bacteria of four weevil species (Coleoptera: Curculionidae) based on analysis of 16S ribosomal DNA. Insect Biochem Mol Biol 22:415–421
7. Clark MA, Baumann L, Munson MA, Baumann P, Campbell BC, Duffus JE, Osborne LS, Moran NA (1992) The eubacterial endosymbionts of whiteflies (Homoptera: Aleyrodoidea) constitute a lineage distinct from the endosymbionts of aphids and mealybugs. Curr Microbiol 25:119–123
8. Cohen S, Duffus JE, Liu HY (1992) A new *Bemisia tabaci* biotype in the Southwestern United States and its role in silverleaf of squash and transmission of lettuce infectious yellows virus. Phytopathology 82:86–90
9. Duffus JE, Flock RA (1982) Whitefly-transmitted disease complex of the desert southwest. Calif Agric 36 (11 & 12): 4–6
10. Gerling D (ed.) (1990) Whiteflies: their bionomics, pest status and management. Newcastle upon Tyne: Athenaeum Press
11. Hendriks L, De Baere R, Van Broeckhoven C, De Wachter R (1988) Primary and secondary structure of the 18S ribosomal RNA of the insect species *Tenebrio molitor*. FEBS Lett 232:115–120

12. Hennig W (1980) Insect phylogeny (edition with revisionary notes by D Schlee). New York: Wiley and Sons
13. Kwon O-Y, Ogino K, Ishikawa H (1991) The longest 18S ribosomal RNA ever known: nucleotide sequence and presumed secondary structure of the 18S rRNA of the pea aphid, *Acyrthosiphon pisum*. Eur J Biochem 202:827–833
14. Munson MA, Baumann P, Clark MA, Baumann L, Moran NA, Voegtlin DJ, Campbell BC (1991) Evidence for the establishment of aphid–eubacterium endosymbiosis in an ancestor of four aphid families. J Bacteriol 173:6321–6324
15. Munson MA, Baumann P, Moran NA (1992) Phylogenetic relationships of the endosymbionts of mealybugs (Homoptera: Psedococcidae) based on 16S rDNA sequences. Mol Phylogen Evol 1:26–30
16. Perring TM, Cooper A, Kazmer DJ (1992) Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. J Econ Entomol, in press
17. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn, GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491
18. Tautz D, Hancock JM, Webb DA, Tautz C, Dover GA (1988) Complete sequences of the rRNA genes of *Drosophila melanogaster*. Mol Biol Evol 5:366–376
19. Tremblay E (1959) Osservazioni sulla simbiosi endocellulare di alcuni Aleyrodidae (*Bemisia tabaci* Gennad., *Aleurolobus olivinus* Silv., *Trialeurodes vaporariorum* West.). Boll Lab Entomol Agric "F. Silvestry" Portici 27:210–246